

WHAT IS CLAIMED IS:

1. A method for producing mammalian  
5 proteins comprising:  
growing mammalian secondary expression host  
cells comprising multiple copies of an amplifiable  
region comprising a target gene heterologous to said  
secondary expression host and expressing a protein of  
10 interest and an amplifiable gene, whereby said target  
gene is expressed and said protein is produced;  
wherein said secondary host expression cells  
are produced by the method comprising:  
transforming primary mammalian cells  
15 comprising said target gene with a construct  
comprising an amplifiable gene and at least one  
flanking region of a total of at least about 150 bp  
homologous with a DNA sequence at the locus of the  
coding region of said target gene to provide  
20 amplification of said target gene, wherein said  
amplifiable gene is at a site which does not  
interfere with the expression of said target gene,  
whereby said construct becomes homologously integrated  
into the genome of said primary cells to define an  
25 amplifiable region;  
selecting for primary cells comprising said  
construct by means of said amplifiable gene or other  
marker present in said construct;  
isolating DNA portions of said genome from  
30 said primary cells, wherein said portions are large  
enough to include all of said amplifiable region;  
transforming secondary expression host cells  
with said primary cell DNA portions and cloning said  
transformed secondary expression host cells to  
35 produce clones of said secondary expression host  
cells differing in said DNA portions present in said  
secondary expression host cells;

selecting clones of said mammalian secondary expression host cells comprising said amplifiable region; and

5 amplifying said amplifiable region by means of an amplifying agent, wherein said amplifying is prior to said isolating or after said selecting and prior to said growing.

2. A method according to Claim 1, wherein said amplifiable gene is a mammalian DHFR gene.

10 3. A method according to Claim 1, wherein said portions are metaphase chromosomes.

4. A method according to Claim 1, wherein said portions are restriction fragments.

15 5. A method according to Claim 1, wherein said primary cells are human cells.

6. A method according to Claim 5, wherein said human cells are fibroblast cells.

20 7. A method according to Claim 1, wherein said construct comprises a biocidal marker providing resistance to a biocide for said primary host cells.

8. A method for producing mammalian proteins comprising:

25 transforming mammalian primary mammalian cells comprising said target gene with a construct comprising an amplifiable gene and at least one flanking region of at least about 150 bp homologous with a DNA sequence within 50 kb of the coding region of said target gene, wherein said amplifiable gene is at a site which does not interfere with the

30 expression of said target gene, whereby said construct becomes homologously integrated into the genome of said primary cells to define an amplifiable region comprising said amplifiable gene and said target gene in said genome;

35 selecting for primary cells comprising said construct by means of said amplifiable gene or other marker present in said construct;

isolating DNA portions of said genome from  
said primary cells, wherein said portions are large  
enough to include all of said amplifiable region;

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5 transforming mammalian secondary expression  
host cells with said primary cell DNA portions,  
wherein said secondary expression host cells are of a  
different species from said primary host cells, and  
cloning said transformed secondary expression host  
cells to produce clones of said secondary expression  
10 host cells differing in said DNA portions present in  
said secondary expression host cells;

selecting clones of said mammalian secondary  
expression host cells comprising said amplifiable  
region;

15 amplifying said amplifiable region by means  
of an amplifying agent, wherein said amplifying is  
prior to said isolating or after said selecting; and

growing said secondary expression host cells  
comprising multiple copies of said amplifiable region,  
20 whereby said target gene is expressed and said protein  
is produced.

9. A method according to Claim 8, wherein  
said amplifying is with said secondary expression host  
cells.

25 10. A method according to Claim 8, wherein  
said primary cells are human cells.

11. A method according to Claim 10, wherein  
said human cells are diploid fibroblast cells.

30 12. A method according to Claim 8, wherein  
said amplifiable gene is a mutated DHFR gene having a  
higher Km than the wild-type gene.

13. A method according to Claim 12, wherein  
said secondary host expression cell is DHFR deficient.

35 14. A method according to Claim 8, wherein  
said construct further comprises a marker gene  
separated from said amplifiable region by an  
homologous flanking region.

isolating DNA portions of said genome from  
said primary cells, wherein said portions are large  
enough to include all of said amplifiable region;

transforming mammalian secondary expression  
5 host cells with said primary cell DNA portions,  
wherein said secondary expression host cells are of a  
different species from said primary host cells, and  
cloning said transformed secondary expression host  
cells to produce clones of said secondary expression  
10 host cells differing in said DNA portions present in  
said secondary expression host cells;

selecting clones of said mammalian secondary  
expression host cells comprising said amplifiable  
region; and amplifying said amplifiable region by  
15 means of an amplifying agent, wherein said amplifying  
is either prior to said isolating or after said  
selecting.

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20 21. A method according to Claim 20, wherein  
said amplifying is with said secondary expression host  
cells.

22. A method according to Claim 20, wherein  
said primary cells are human cells.

23. A method according to Claim 22, wherein  
said human cells are diploid fibroblast cells.

25 24. A method according to Claim 20, wherein  
said amplifiable gene is a mutated DHFR gene having a  
higher Km than the wild-type gene.

25. A method according to Claim 24, wherein  
said secondary host expression cell is DHFR deficient.

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